Retrospective Cohort Study

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Association of severity in the grading of microvascular invasion with long-term oncological prognosis after liver resection for early-stage hepatocellular carcinoma: a multicenter retrospective cohort study from a hepatitis B virus-endemic area

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Background: The presence of microvascular invasion (MVI) is a significant malignant pathological feature related to recurrence and survival after liver resection for hepatocellular carcinoma (HCC). This study aimed to investigate the relationship between the severity in the grading of MVI and long-term oncological outcomes in patients with early-stage HCC.

Methods: A retrospective study was conducted on a prospectively maintained multicenter database on patients who underwent curative resection for Barcelona Clinic Liver Cancer stage 0/A HCC between 2017 and 2020. Patients were classified into three groups according to the severity in the grading of MVI: M0 (no MVI), M1 (1–5 sites of MVI occurring $\leq$1 cm away from the tumor), and M2 (>5 sites occurring $\leq$1 cm and/or any site occurring >1 cm away from the tumor). Recurrence-free survival (RFS) and overall survival (OS) were compared among the groups.

Results: Of 388 patients, M0, M1, and M2 of the MVI gradings were present in 223 (57.5%), 118 (30.4%), and 47 (12.1%) patients, respectively. The median OS and RFS in patients with M0, M1, and M2 were 61.1, 52.7, and 27.4 months; and 43.0, 29.1, and 13.1 months (both $P<0.001$), respectively. Multivariable analyses identified both M1 and M2 to be independent risk factors for OS [hazard ratio (HR): 1.682, $P=0.003$; and HR: 3.570, $P<0.001$] and RFS (HR: 1.550, $P=0.037$; and HR: 2.256, $P<0.001$).

Conclusion: The severity in the grading of MVI was independently associated with recurrence and survival after HCC resection. Patients with the presence of MVI, especially those with a more severe MVI grading (M2), require more stringent recurrence surveillance and/or active adjuvant therapy against recurrence.

Keywords: hepatocellular carcinoma, microvascular invasion, recurrence, surgery, survival

Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide and constitutes a major public health burden[1,2]. For patients with early-stage HCC, defined as Barcelona Clinic Liver Cancer (BCLC) stage 0/A, surgical resection is a well-accepted treatment that can provide a chance of long-term cure[3,4]. Recent advances in operative techniques and perioperative management have improved the short-term and long-term outcomes after surgical resection for HCC, with a perioperative mortality rate of less than 3% and a 5-year survival rate for early-stage HCC of up to 60–70%[5–8]. However,

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long-term survival remains unsatisfactory because of the high incidence of cancer recurrence, which is the main cause of poor prognosis. Postoperative recurrence within 5 years of liver resection for early-stage HCC has been reported to range from 30 to 50%[7–9]. To improve surgical outcomes of HCC patients at high risk of recurrence, risk factors need to be identified so that more stringent recurrence surveillance and/or active adjuvant therapy can be offered, especially for patients with tumors at an 'early stage' based on the commonly used clinical staging systems, so as to improve treatment outcomes of these patients.

Numerous studies have demonstrated that the most critical determinant of postoperative recurrence of HCC is the presence of microvascular invasion (MVI), which has been widely recognized to be closely related to the high tumor aggressiveness of HCC[10–14]. Our team and other researchers have reported that the incidences of MVI were between 22 and 46% in surgically resected specimens of early-stage HCC, while the adjusted increased risk of postoperative recurrence after liver resection for these tumors was up to 80%[8,12–14]. MVI can only be confirmed by histopathological microscopic examination of resected surgical specimens, and MVI is generally defined as the presence of a cluster of tumor cells in microscopic vessels located in the peritumoral liver[15–17]. However, the severity in the grading of MVI, especially the number and location of MVI as detected by microscopy in the peritumoral liver on HCC recurrence and survival after liver resection, has rarely been reported[18,19].

To standardize and refine histopathological diagnoses of MVI in HCC-resected specimens, the Liver Cancer Pathology Group of China (LCPGC) proposed a 7-point baseline sample collection and the three-tiered MVI grading system (MVI-TTG) in 2015. This system was based on the location and number of MVI detected on microscopy in the peritumoral liver[20]. The MVI-TTG system divides the severity in the grading of MVI into three categories: M0, M1, and M2, to replace the traditional two categories of presence or absence of MVI. The aim of this multicenter study is to identify whether the severity in the grading of MVI using this MVI-TTG system is associated with long-term postoperative oncologic prognosis in survival and recurrence in patients after liver resection with curative intent for early-stage HCC.

HIGHLIGHTS

- The severity in the grading of microvascular invasion (MVI) based on the three-tiered MVI grading system was independently associated with recurrence and survival after hepatocellular carcinoma resection.
- Patients with the presence of MVI, especially those with a more severe MVI grading (M2), require more stringent recurrence surveillance and/or active adjuvant therapy against recurrence after curative resection for early-stage hepatocellular carcinoma.

Methods

Study population

The data of this retrospective study was obtained from a prospectively collected database on consecutive patients with HCC who underwent curative-intent liver resection from January 2017 to December 2020 at three hepatobiliary centers in China (Eastern Hepatobiliary Surgery Hospital of Shanghai, Mengchao Hepatobiliary Hospital, and Zhejiang Provincial People's Hospital). Patients were excluded if they: were less than 18 years old; had recurrent HCC; had intermediate-stage or advanced-stage HCC (BCLC stage B/C); received preoperative anti-HCC treatment; underwent R1 or R2 resection; developed postoperative early death within 90 days of surgery; were lost to follow-up within 6 months of surgery; and had missing important prognostic variables including the MVI status. The study was performed in accordance with the Declaration of Helsinki and the Ethical Guidelines for Clinical Studies (No. EHBHKY2020-01-093). Written informed consent for the relevant procedures and the use of data for research purposes were obtained from all the patients before surgery. This retrospective study was registered with ResearchRegistry.com (Unique Identification Number: researchregistry8040) and has been reported in line with the STROCSS (Strengthening the reporting of cohort studies in surgery) criteria[21].

Baseline characteristics and clinical variables

Clinicopathological variables, including age, gender, American Society of Anesthesiologists (ASA) score, BMI, diabetes mellitus, hepatitis B virus infection, hepatitis C virus infection, cirrhosis, portal hypertension, Child–Pugh grading, aspartate aminotransferase (AST) level, alanine aminotransferase (ALT) level, and α-fetoprotein (AFP) level, maximum tumor size, tumor number, MVI, satellite nodules, tumor encapsulation, and tumor differentiation were reviewed. Cirrhosis was confirmed by histopathological examination. According to the WHO classification, BMI was formulated by bodyweight (kg)/height^2 (m^2), and overweight was defined as BMI greater than 24.9 kg/m^2[22]. Portal hypertension was defined as either presence of esophageal varices or splenomegaly with a decreased platelet count (100×10^9/l or less). Multiple tumors were defined as two or more tumor nodules. Since the present study focuses on BCLC early-stage HCC, multiple tumors actually referred to two or three tumor nodules (within Milan criteria). Perioperative outcomes including intraoperative blood loss, intraoperative blood transfusion, extent of hepatectomy (minor or major), type of resection (anatomical or nonanatomical), and resection margin were analyzed. Major hepatectomy was defined as the resection of three or more Couinaud segments, while minor hepatectomy as a resection of fewer than three segments. Anatomical resection was defined by the Brisbane 2000 nomenclature of liver anatomy and resections[23].

Sampling protocol and severity in the grading of MVI

All surgical specimens were sampled according to the 7-point baseline sample collection protocol[20]. Four tissue specimens were sampled at 12, 3, 6, and 9 o'clock positions at the junction of the tumor and the adjacent liver tissues in a 1 : 1 ratio, together with one specimen sampled at the intratumoral zone and two specimens sampled within 1 cm from the tumor capsule and over 1 cm from the tumor capsule or tumor margin. The severity in the grading of MVI was determined by two experienced pathologists based on the Chinese MVI-TTG system[20]. MVI was defined as the presence of a cluster of tumor cells lined by the endothelium in vessels under microscopy. Severity in the grading of MVI was graded as M0 (no MVI), M1 (1–5 sites of MVI occurring in the tumor-adjacent liver tissue $\leq$ 1.0 cm away from the main tumor),

M2 (> 5 sites of MVI occurring in the tumor-adjacent liver tissue ≤ 1.0 cm, and/or any MVI existing in distant liver tissue > 1.0 cm away from the main tumor).

Follow-up

All patients were followed up regularly after discharge from the hospital. Ultrasonography, serum AFP level, contrast-enhanced computed tomography (CT), or MRI were performed at a 2–3 monthly interval for the first 6 months after surgery, 3–4 monthly intervals for the next 18 months, and then 3–6 monthly intervals thereafter. Tumor recurrence was clinically suspected with a progressive elevation of serum AFP levels and/or ultrasonographic detection of a new hepatic lesion. The diagnosis of recurrence was made with a dynamic CT scan or MRI, which demonstrated contrast enhancement in the arterial phase and wash-out in the venous phase, or with hepatic angiography, which disclosed high tumor vascularity. Treatment options for patients with tumor recurrence included repeat resection, local ablation, liver transplantation, transcatheter arterial chemoembolization, systemic therapy, or supportive care based on recurrence patterns, liver functional reserve, and patients' general conditions. This treatment strategy for recurrence was consistently applied and relatively standardized across all the participating hospitals.

Study endpoints and statistical analysis

As this is a retrospective observational study, no formal sample size calculation was performed; instead, all available patients' fulfilling inclusion and exclusion criteria were considered for the present study. The two study endpoints were overall survival (OS) and recurrence-free survival (RFS). OS was calculated from the date of surgery to either the date of death caused by any reason or the date of the last follow-up, while RFS was calculated from the date of surgery to the date of diagnosis of initial recurrence or the date of death caused by any reason or the last follow-up. Continuous variables and categorical variables were expressed as mean ± SD and number (percentage), respectively. Student's t test and Mann–Whitney U test were used for the comparison of continuous variables, while the χ^2 test or Fisher's exact test were used for categorical variables, as appropriate. OS and RFS were calculated by the Kaplan–Meier method, and differences among groups were compared with the log-rank test. The univariable and multivariable Cox proportional hazard regression model was used to identify independent variables that were associated with OS and RFS. Variables with a P value of less than 0.10 on univariable analysis were subjected to a multivariable Cox regression model using a forward stepwise variable selection. All P values were two-tailed, with a level of less than 0.05 being considered statistically significant. Statistical analyses were performed using the SPSS software version 25.0 (SPSS, Chicago, Illinois, USA).

Results

Comparisons of clinical characteristics

During the study period, 905 patients underwent liver resection with curative intent for HCC at the three hepatobiliary centers in China. Using the predetermined inclusion and exclusion criteria, 388 patients with early-stage (BCLC stage 0/A) HCC were enrolled in the study (Fig. 1). For the whole cohort, 347 (89.4%) patients were males and 41 (10.6%) were females. The median age was 51.0 (range 21–79) years. Most patients (91.5%) had chronic hepatitis B virus (HBV) infection, and only 14 patients (3.6%) had chronic hepatitis C virus (HCV) infection. In addition, 80.7% of these patients had cirrhosis and 21.2% had clinically significant portal hypertension. Postoperative microscopic examination of the resected surgical specimens showed the severity in the grading of MVI to be M0, M1, and M2 in 223 (57.5%), 118 (30.4%), and 47 (12.1%) patients, respectively.

Table 1 shows the clinical characteristics in the M0, M1, and M2 groups of patients. Significant differences existed between the M0 and M1 groups in serum AST level ($P = 0.045$), ALT level ($P = 0.003$), AFP level ($P = 0.001$), satellite nodules ($P < 0.001$), tumor encapsulation ($P < 0.001$), tumor differentiation ($P < 0.001$), and extent of hepatectomy ($P = 0.005$). There were also significant differences between the M0 and M2 groups in ASA score ($P = 0.031$), multiple tumors ($P < 0.001$), satellite nodules ($P < 0.001$), tumor encapsulation ($P < 0.001$), tumor differentiation ($P = 0.001$), and intraoperative blood loss ($P = 0.003$). In addition, there were significant differences between the M1 and M2 groups in multiple tumors ($P < 0.001$), satellite nodules ($P < 0.001$), tumor encapsulation ($P = 0.041$), intraoperative blood loss ($P = 0.009$), and type of resection ($P = 0.045$).

Comparisons of long-term oncologic outcomes

With median follow-up times of 46.8, 44.9, and 25.8 months for patients in the M0, M1, and M2 groups, respectively; 74, 61, and 33 patients in each group died ($P < 0.001$), and 105, 69, and 35 patients in each group developed HCC recurrence ($P = 0.001$), respectively. Comparisons of the long-term postoperative oncological outcomes in the three groups are shown in Table 2. In the M0, M1, and M2 groups, the overall recurrence rates were 47.1, 58.5, and 74.5%, respectively, with a close to a significant difference between the M0 and M1 groups ($P = 0.053$) and between the M1 and M2 groups ($P = 0.074$). The corresponding mortality rates were 33.2, 51.7, and 70.2%, respectively, with a significant difference between the M0 and M1 groups ($P = 0.001$) and between the M1 and M2 groups ($P = 0.037$). The median OS and RFS of the M0, M1, and M2 groups were 61.1 and 43.0 months, 52.7 and 29.1 months, and 27.4 and 13.1 months, respectively. There were significant differences in OS and RFS between the M0 and M1 groups ($P = 0.001$ and 0.033) and between the M1 and M2 groups ($P = 0.001$ and 0.005).

The 1-year, 3-year, and 5-year OS rates for patients in the M0, M1, and M2 groups were 99.1, 81.9, and 57.5%; 92.4, 63.7, and 32.1%; and 87.2, 38.8, and 24.4%, respectively (Fig. 2 and Table 2). Compared with the M0 group, patients in both the M1 and M2 groups showed a decreased OS rate after curative liver resection for early-stage HCC (HR: 1.768, 95% CI: 1.259–2.483, $P = 0.001$; and HR: 3.726, 95% CI: 2.464–5.634, $P < 0.001$, respectively) (Table 3).

The 1-year, 3-year, and 5-year RFS rates for patients in the M0, M1, and M2 groups were 82.5, 56.1, and 46.0%; 70.3, 46.4, and 35.5%; and 55.3, 25.0, and 22.0%, respectively (Fig. 3 and Table 2). Compared with the M0 group, patients in both the M1 and M2 groups showed a decreased RFS rate after curative liver resection for early-stage HCC (HR: 1.383, 95%

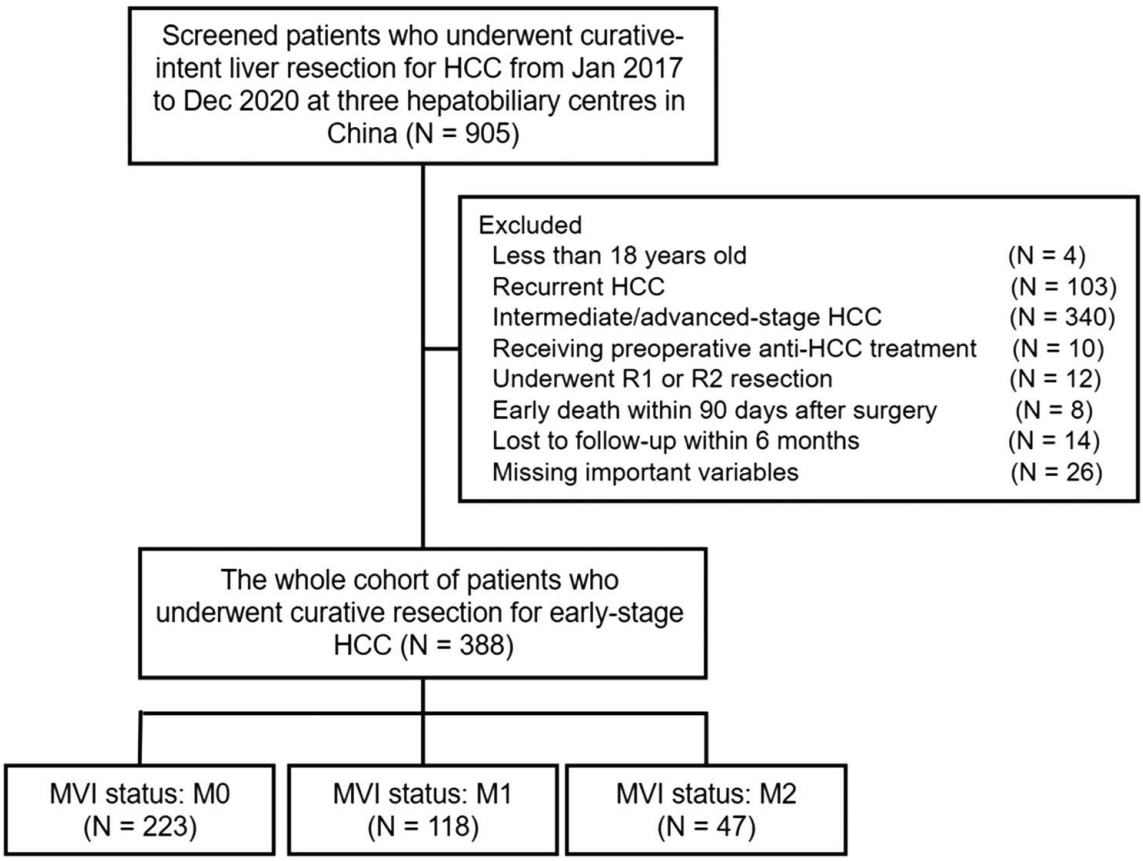


Figure 1. Selection of the study population. HCC, hepatocellular carcinoma; MVI, microvascular invasion.

CI: 1.025–1.866, $P = 0.034$; and HR: 2.573, 95% CI: 1.762–3.758, $P < 0.001$, respectively) (Table 4).

Univariable and multivariable analysis of OS and RFS

The results of the univariable and multivariable Cox proportional hazard regression analyses of OS and RFS after curative liver resection for early-stage HCC are shown in Tables 3 and 4. Multivariable analyses identified that when compared to the M0 group, both the M1 and M2 groups were independently associated with poorer OS (HR: 1.682, 95% CI: 1.188–2.382, $P = 0.003$; and HR: 3.570, 95% CI: 2.346–5.431, $P < 0.001$, respectively) and poorer RFS (HR: 1.550, 95% CI: 1.026–2.343, $P = 0.037$; and HR: 2.256, 95% CI: 1.508–3.377, $P < 0.001$), respectively.

In addition to the severity in the grading of MVI (M1 and M2), other independent risk factors of OS included cirrhosis (HR: 1.705, 95% CI: 1.078–2.696, $P = 0.022$), Child–Pugh grade B (HR: 1.815, 95% CI: 1.120–2.942, $P = 0.016$), preoperative AFP level greater than 400 μg/l (HR: 1.494, 95% CI: 1.083–2.061, $P = 0.014$), tumor size greater than 5 cm (HR: 1.492, 95% CI: 1.094–2.034, $P = 0.011$), and resection margin less than 1 cm (HR: 1.905, 95% CI: 1.389–2.614, $P < 0.001$) (Table 3). The other independent risk factors of RFS included tumor size greater than 5 cm (HR: 1.331, 95% CI: 1.014–1.748, $P = 0.040$), incomplete tumor encapsulation (HR: 1.369, 95% CI: 1.023–1.831, $P = 0.034$), intraoperative blood transfusion (HR: 1.661, 95% CI: 1.144–2.431, $P = 0.008$), and resection margin less than 1 cm (HR: 1.556, 95% CI: 1.181–2.051, $P = 0.002$) (Table 4).

Discussion

Numerous studies have identified that the presence of MVI is an aggressive biological characteristic of HCC, and it has been shown to be one of the most critical risk factors for postoperative recurrence and survival after HCC resection[12,24]. The present study further confirmed that not only the presence of MVI but the severity in the grading of MVI (the number and location of MVI as detected by microscopy) are associated with long-term recurrence and survival after liver resection for HCC. Based on the MVI-TTG system proposed by the LCPGC, the severity in the grading of MVI can be divided into three categories: M0, M1, and M2 combining the number of MVIs detected under microscopy with the distance of MVI (over or under 1 cm) from the main tumor. This system is a balance which is based on the huge experience in the management of HCC patients with MVI in China, technical practicality, and convenience for pathologists to produce a standardized report in clinical practice, with a pathological report which can facilitate clinicians to better understand the significance of the severity in the grading of MVI so that these clinicians can transmit relevant information to patients. In the present study, M2, which represented the most severe grade in the MVI status, increased the multivariable-adjusted HRs of postoperative death and recurrence by over 250 and 125%,

Table 1

Clinical characteristics in the three groups according to different severity in the grading of microvascular invasion on the pathological examination.

	M0 ($n=223$)	M1 ($n=118$)	M2 ($n=47$)	*P* (M0 vs. M1)	*P* (M0 vs. M2)	*P* (M1 vs. M2)
Age (years)[a]	51.2 ± 10.2	50.4 ± 10.9	50.2 ± 11.4	0.491	0.537	0.910
Male sex	202 (90.6)	105 (89.0)	40 (85.1)	0.639	0.392	0.491
ASA score > 2	18 (8.1)	18 (15.3)	9 (19.1)	0.062	0.031	0.542
BMI (kg/m^2)[a]	23.4 ± 2.9	23.8 ± 3.3	23.6 ± 3.1	0.316	0.776	0.703
Diabetes mellitus	15 (6.7)	9 (7.6)	3 (6.4)	0.757	1.000	1.000
HBsAg-positive	205 (91.9)	108 (91.5)	42 (89.4)	0.897	0.775	0.892
Anti-HCV-positive	7 (3.1)	5 (4.2)	2 (4.3)	0.830	1.000	1.000
Cirrhosis	180 (80.7)	93 (78.8)	40 (85.1)	0.676	0.481	0.356
Portal hypertension	47 (21.1)	28 (23.7)	11 (23.4)	0.574	0.724	0.965
Child–Pugh grade B	19 (8.5)	6 (5.1)	7 (14.9)	0.247	0.283	0.052
AST > 40 U/l	56 (25.1)	42 (35.6)	13 (27.7)	0.045	0.716	0.329
ALT > 40 U/l	66 (29.6)	54 (45.8)	18 (38.3)	0.003	0.242	0.383
AFP level > 400 μg/l	56 (25.1)	50 (42.4)	17 (36.2)	0.001	0.121	0.464
Tumor size > 5.0 cm	87 (39.0)	57 (48.3)	22 (46.8)	0.098	0.322	0.962
Multiple tumors	11 (4.9)	2 (1.7)	42 (89.4)	0.235	< 0.001	< 0.001
Satellite nodules	9 (4.0)	25 (21.2)	30 (63.8)	< 0.001	< 0.001	< 0.001
Tumor encapsulation						
Complete	158 (70.9)	43 (36.4)	9 (19.1)	< 0.001	< 0.001	0.041
None or incomplete	65 (29.1)	75 (63.6)	38 (80.9)			
Tumor differentiation						
Poorly	86 (38.6)	75 (63.6)	30 (63.8)	< 0.001	0.001	0.904
Well or moderately	137 (61.4)	43 (36.4)	17 (36.2)			
Intraoperative blood loss (> 600 ml)	28 (12.6)	15 (12.7)	14 (29.8)	0.967	0.003	0.009
Intraoperative blood transfusion	24 (10.8)	13 (11.0)	9 (19.1)	0.943	0.111	0.165
Extent of hepatectomy						
Minor	207 (92.8)	98 (83.1)	41 (87.2)	0.005	0.327	0.506
Major	16 (7.2)	20 (16.9)	6 (12.8)			
Type of resection						
Anatomical	40 (17.9)	27 (22.9)	4 (8.5)	0.274	0.112	0.045
Nonanatomical	183 (82.1)	91 (77.1)	43 (91.5)			
Resection margin						
≤ 1.0 cm	107 (48.0)	54 (45.8)	27 (57.4)	0.696	0.238	0.175
> 1.0 cm	116 (52.0)	64 (54.2)	20 (42.6)			

Values in parentheses are percentages unless indicated otherwise.
[a]Values are mean (SD).
AFP, α-fetoprotein; ALT, alanine aminotransferase; ASA, American Society of Anesthesiologists; AST, aspartate aminotransferase; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

Table 2

Long-term postoperative oncologic outcomes in the three groups according to different severity in the grading of microvascular invasion on the pathological examination.

	M0 ($n=223$)	M1 ($n=118$)	M2 ($n=47$)	*P* (M0 vs. M1)	*P* (M0 vs. M2)	*P* (M1 vs. M2)
Death during follow-up	74 (33.2)	61 (51.7)	33 (70.2)	0.001	< 0.001	0.037
Recurrence during follow-up	105 (47.1)	69 (58.5)	35 (74.5)	0.053	0.001	0.074
Pattern of initial recurrence						
Intrahepatic only	85 (38.1)	45 (38.1)	20 (42.6)	0.037	0.009	0.648
Extrahepatic only	6 (2.7)	11 (9.3)	8 (17.0)			
Intrahepatic and extrahepatic	14 (6.3)	13 (11.0)	7 (14.9)			
OS (months)a	61.1 (NR)	52.7 (44.4–61.0)	27.4 (19.8–35.0)	0.001	< 0.001	0.001
1-year rate (%)	99.1	92.4	87.2			
3-year rate (%)	81.9	63.7	38.8			
5-year rate (%)	57.5	32.1	24.4			
RFS (months)[a]	43.0 (NR)	29.1 (13.8–44.4)	13.1 (9.9–16.7)	0.033	< 0.001	0.005
1-year rate (%)	82.5	70.3	55.3			
3-year rate (%)	56.1	46.4	25.0			
5-year rate (%)	46.0	35.5	22.0			

Values in parentheses are percentages unless indicated otherwise.
[a]Values are median (95% CIs).
NR, not reached; OS, overall survival; RFS, recurrence-free survival.

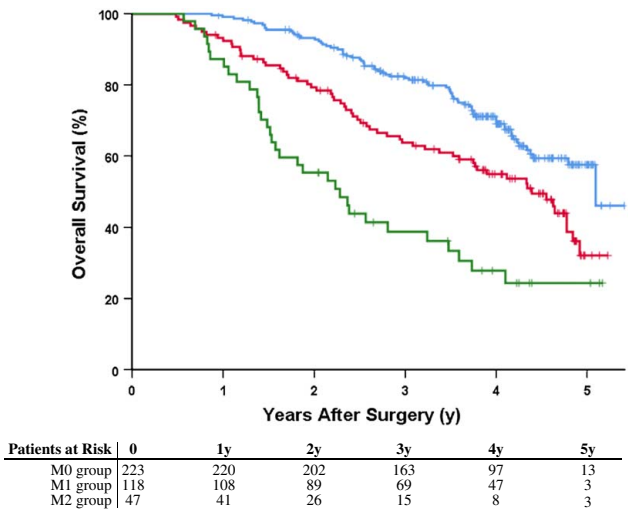


Figure 2. Kaplan–Meier curves of overall survival (OS) among patients with different severity in the grading of microvascular invasion (M0, M1, and M2). $P = 0.001$ (M0 vs. M1), $P = 0.001$ (M1 vs. M2), and $P < 0.001$ (M0 vs. M2) (log-rank test).

respectively, when compared with the M0 status. Furthermore, M1 was also shown to be an independent risk factor associated with poorer OS (increased multivariable HR: 68%) and RFS (increased multivariable HR: 55%). The results of this study clearly showed that the prognostic significance of MVI lay not only in its presence or absence but also in the severity in the grading of MVI, which has important clinical implications on postoperative recurrence surveillance and antirecurrence strategies in HCC patients defined by commonly used clinical stagings to be in early HCC stages.

In order to clearly identify the relationship between severity in MVI grading and long-term oncologic prognosis, patients who died within 3 months after surgery mostly due to postoperative complications ($n = 8$) and patients who were lost to follow-up within 6 months after surgery ($n = 14$) were excluded from the analytic cohort. As we believe, this reasonable inclusion and exclusion criteria will make the conclusion of the present study more accurate and convincing.

As shown in Table 1, there were close relationships between the severity of grading of MVI with a few tumor-related characteristics, which would also have influenced the long-term outcomes of patients with HCC after liver resection, such as preoperative AFP level, tumor size, tumor number, the presence of satellite nodules, tumor encapsulation, and tumor differentiation. After multivariate analyses to adjust the influence of confounding risk factors, the results identified that the severity in the grading of MVI (both M1 and M2) and tumor size were independent risk factors of both OS and RFS after liver resection for early-stage HCC.

As MVI is not evenly distributed in tumor-adjacent liver tissues[15,16], more sampling sites should result in a more thorough examination of tissues and increase the likelihood of

Table 3

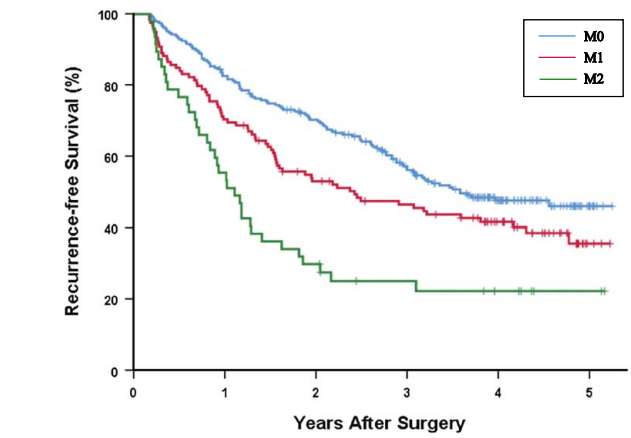
Univariable and multivariable Cox regression analysis of risk factors for overall survival (OS) after curative resection of early-stage hepatocellular carcinoma.

Variables	Comparisons	UV HR (95% CI)	UV *P*	MV HR (95% CI)	MV *P*[a]
Age	> 60 vs. ≤ 60 years	1.069 (0.727–1.572)	0.735		
Sex	Male vs. female	0.896 (0.556–1.444)	0.651		
ASA score	> 2 vs. ≤ 2	1.391 (0.886–2.182)	0.151		
Overweight	Yes vs. no	1.348 (0.979–1.855)	0.067	NA	0.171
Diabetes mellitus	Yes vs. no	1.259 (0.741–2.141)	0.395		
HBsAg-positive	Yes vs. no	0.925 (0.535–1.598)	0.779		
Anti-HCV-positive	Yes vs. no	1.343 (0.629–2.868)	0.445		
Cirrhosis	Yes vs. no	1.978 (1.263–3.100)	0.003	1.705 (1.078–2.696)	0.022
Portal hypertension	Yes vs. no	1.346 (0.954–1.900)	0.091	NA	0.561
Child–Pugh grade	B vs. A	1.928 (1.206–3.081)	0.006	1.815 (1.120–2.942)	0.016
AST	> 40 vs. ≤ 40 U/l	1.167 (0.843–1.616)	0.353		
ALT	> 40 vs. ≤ 40 U/l	1.082 (0.793–1.478)	0.618		
Preoperative AFP level	> 400 vs. ≤ 400 μg/l	1.616 (1.183–2.208)	0.003	1.494 (1.083–2.061)	0.014
Tumor size	> 5.0 vs. ≤ 5.0 cm	1.675 (1.237–2.269)	0.001	1.492 (1.094–2.034)	0.011
Multiple tumors	Yes vs. no	2.380 (1.644–3.446)	< 0.001	NA	0.184
Microvascular invasion grade	M1 vs. M0	1.768 (1.259–2.483)	0.001	1.682 (1.188–2.382)	0.003
	M2 vs. M0	3.726 (2.464–5.634)	< 0.001	3.570 (2.346–5.431)	< 0.001
Satellite nodules	Yes vs. no	2.178 (1.530–3.100)	< 0.001	NA	0.536
Incomplete tumor encapsulation	Yes vs. no	2.047 (1.504–2.784)	< 0.001	NA	0.058
Poorly tumor differentiation	Yes vs. no	0.996 (0.736–1.348)	0.980		
Intraoperative blood loss	> 600 vs. ≤ 600 ml	1.617 (1.105–2.368)	0.013	NA	0.262
Intraoperative blood transfusion	Yes vs. no	1.918 (1.277–2.881)	0.002	NA	0.077
Extent of hepatectomy	Major vs. minor	1.388 (0.884–2.177)	0.154		
Type of resection	Anatomical vs. nonanatomical	0.826 (0.547–1.248)	0.364		
Resection margin	< 1.0 vs. ≥ 1.0 cm	1.937 (1.422–2.639)	< 0.001	1.905 (1.389–2.614)	< 0.001

Values in parentheses are 95% CIs.

[a]Variables significant at $P < 0.1$ in the univariate analysis were entered into the multivariate analysis.

AFP, α-fetoprotein; ALT, alanine aminotransferase; ASA, American Society of Anesthesiologists; AST, aspartate aminotransferase; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HR, hazard ratio; MV, mutlivariable; NA, not available; UV, univariable.



Patients at Risk	0	1y	2y	3y	4y	5y
M0 group	223	183	151	108	55	8
M1 group	118	83	59	49	33	3
M2 group	47	26	14	9	6	2

Figure 3. Kaplan–Meier curves of recurrence-free survival (RFS) among patients with different severity in the grading of microvascular invasion (M0, M1, and M2). $P = 0.033$ (M0 vs. M1), $P = 0.005$ (M1 vs. M2), and $P < 0.001$ (M0 vs. M2) (log-rank test).

detecting MVI. Torbenson[25] proposed a 3-point sampling protocol for liver cancer, while Roayaie *et al.*[16] took over 10 noncancerous tissue blocks for MVI detection. The sampling number, however, cannot be increased indefinitely, considering the technical practicality of pathologists. Recently, Kuang *et al.* conducted both a retrospective and prospective study on the impact of the number of sample sites on MVI status and long-term outcomes. They recommended a threshold of four, six, eight, and eight sampling sites for solitary tumors measuring 1.0–3.0 cm, 3.1–4.9 cm, over 5.0 cm, and multiple tumors, respectively[19]. In our study, patients were sampled according to the 7-point baseline sampling protocol proposed by LCPGC, which is a standard HCC sampling protocol in China for histopathological examination of resected HCC specimens. Considering the relationship among sampling number, sampling site, and MVI detection rate, the 7-point baseline sampling protocol is an optimal balance, and its use has been validated in a large nationwide study in China[26].

The MVI-TTG system, which incorporates MVI number and MVI location, has previously been shown to associate with postoperative recurrence and survival[16,27], and this system was therefore adopted in the present study. When compared with the various other MVI staging systems which focus only on MVI numbers[18] and the number of MVI cells[28], the MVI-TTG system balances the application value in clinical practice and the technical practicality of pathologists by stratifying HCC patients into different risks of recurrence and survival without adding excessive time, cost, and pathologists' workload. Since its proposal in 2015, the MVI-TTG system has been promoted and used in most Chinese hospitals, and it has increased the MVI detection rate by 10%[26]. In this retrospective cohort study, the prognostic value of the MVI-TTG system for early-stage HCC was specially

Table 4

Univariable and multivariable Cox regression analysis of risk factors for recurrence-free survival after curative resection of early-stage hepatocellular carcinoma.

Variables	Comparisons	UV HR (95% CI)	UV *P*	MV HR (95% CI)	MV *P*[a]
Age	> 60 vs. ≤60 years	0.978 (0.694–1.378)	0.899		
Sex	Male vs. female	0.972 (0.625–1.511)	0.899		
ASA score	> 2 vs. ≤2	1.251 (0.836–1.874)	0.276		
Overweight	Yes vs. no	1.147 (0.861–1.528)	0.350		
Diabetes mellitus	Yes vs. no	1.369 (0.855–2.192)	0.192		
HBsAg-positive	Yes vs. no	0.873 (0.545–1.399)	0.574		
Anti-HCV-positive	Yes vs. no	1.546 (0.818–2.919)	0.180		
Cirrhosis	Yes vs. no	1.444 (1.009–2.066)	0.044	NA	0.181
Portal hypertension	Yes vs. no	1.078 (0.791–1.469)	0.635		
Child–Pugh grade	B vs. A	1.646 (1.067–2.539)	0.024	NA	0.082
AST	> 40 vs. ≤40 U/l	1.085 (0.814–1.448)	0.577		
ALT	> 40 vs. ≤40 U/l	1.117 (0.849–1.468)	0.430		
Preoperative AFP level	> 400 vs. ≤400 μg/l	1.397 (1.058–1.845)	0.019	NA	0.111
Tumor size	> 5.0 vs. ≤5.0 cm	1.539 (1.179–2.010)	0.002	1.331 (1.014–1.748)	0.040
Multiple tumors	Yes vs. no	2.019 (1.435–2.839)	<0.001	NA	0.162
Microvascular invasion grade	M1 vs. M0	1.383 (1.025–1.866)	0.034	1.550 (1.026–2.343)	0.037
	M2 vs. M0	2.573 (1.762–3.758)	<0.001	2.256 (1.508–3.377)	<0.001
Satellite nodules	Yes vs. no	1.816 (1.310–2.517)	<0.001	NA	0.221
Incomplete tumor encapsulation	Yes vs. no	1.703 (1.303–2.224)	<0.001	1.369 (1.023–1.831)	0.034
Poorly tumor differentiation	Yes vs. no	0.832 (0.637–1.088)	0.179		
Intraoperative blood loss	>600 vs. ≤600 ml	1.543 (1.091–2.184)	0.014	NA	0.959
Intraoperative blood transfusion	Yes vs. no	1.802 (1.243–2.612)	0.002	1.661 (1.144–2.413)	0.008
Extent of hepatectomy	Major vs. minor	1.066 (0.692–1.643)	0.771		
Type of resection	Anatomical vs. nonanatomical	0.798 (0.553–1.151)	0.227		
Resection margin	<1.0 vs. ≥1.0 cm	1.621 (1.240–2.120)	<0.001	1.556 (1.181–2.051)	0.002

Values in parentheses are 95% CIs.

[a]Variables significant at $P < 0.1$ in the univariate analysis were entered into the multivariate analysis.

AFP, α-fetoprotein; ALT, alanine aminotransferase; ASA, American Society of Anesthesiologists; AST, aspartate aminotransferase; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HR, hazard ratio; MV, mutlivariable; NA, not available; UV, univariable.

studied. The results showed that patients with early-stage HCC could be successfully stratified into different risks of recurrence and survival based on the MVI-TTG system.

The resection margin between HCC tissue and its adjacent nontumorous tissue is the key region where HCC invades, and this is a high-incidence area of MVI and satellite nodules. Previous studies revealed that a resection margin of less than 1 cm is associated with unfavorable OS and RFS, especially for patients undergoing nonanatomical resection[29,30]. In our study, the multivariable analysis identified a resection margin less than 1 cm to be an independent risk factor for poor OS and RFS. Hence, a surgical margin of at least 10 mm in partial hepatectomy is recommended for patients with adequate liver functional reserve. Additionally, a previous randomized controlled trial showed that a resection margin of 2 cm was superior to a margin of 1 cm for long-term tumor-related survival[31]. Obviously, the wider the resection margin, the more complete the tumor is resected, and the less likely for it to recur. However, the determination of how wide to carry out a resection margin should be based on tumor characteristics, surgical safety, and patients' liver function. Further studies are required to determine the optimal width of the resection margin for different patient cohorts.

Considering the high recurrence rate after surgical resection for HCC, antirecurrence therapy has always been a hot topic for clinical research, especially for HCC patients with MVI. Although several treatment options like transarterial chemoembolization, systemic treatments with chemotherapy, tyrosine kinase inhibitors, and immunotherapy have been tested in both the adjuvant and neoadjuvant settings, none of them has been universally accepted and none has been recommended by clinical practice guidelines. The STORM study assessed the efficacy of sorafenib as adjuvant therapy for HCC patients after surgical resection or ablation, but no significant difference was observed in RFS between the treatment and the control groups[32]. One possible explanation for this study to fail to show a positive result of using sorafenib as an adjuvant therapy is the inclusion of patients with different risks of recurrence into the study. Further clinical trials are needed to assess the efficacy of adjuvant therapy for patients with MVI, especially for those with M2 who are at high risk of recurrence and poor survival.

There are several limitations in the present study. First, this is a retrospective study which has its inherent biases. Second, the vast majority of patients in this study had HBV infection. Considering that previous studies have revealed the potential association between HBV and MVI[33,34], validation of the results of the present study to Western groups of patients who mostly have chronic HCV infection, nonalcoholic or alcoholic steatohepatitis is necessary. In addition, due to the nature of the retrospective observational study, the details of imaging methods for detection of recurrence, such as using 1.5 or 3 T MRI imaging, as well as patients' postoperative AFP levels during follow-up were not recorded in this multicenter database, which may hinder the further in-depth understanding of timely detection and prediction of recurrence.

Conclusion

In conclusion, the present study demonstrated that M1 and M2 of the severity in the grading of MVI were both independently associated with poorer OS and RFS after curative resection for early-stage HCC. The MVI classification based on the MVI-TTG system as proposed by the LCPGC could stratify patients into groups with different risks of recurrence and long-term survival. Enhanced recurrence surveillance and potentially effective adjuvant treatments against recurrence are worthy of further studies for patients with 'early-stage' HCC and positivity of MVI with M1 or M2 status.

Ethical approval

Yes. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Third Affiliated Hospital of the Naval Medical University of Shanghai (No. EHBHKY2020-01-093).

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Author contribution

X.-F.X.: data curation, formal analysis, methodology, software and visualization, and writing – original draft; Y.-K.D.: data curation, methodology, software and visualization, and writing – original draft; Y.-Y.Z.: conceptualization, data curation, methodology, supervision, and writing – review and editing; C.L.: data curation, methodology, and software and visualization; F.-W.L.: formal analysis, methodology, and software and visualization; L.-Y.S.: data curation; H.W.: data curation; K.-Y.L.: data curation; L.-Q.Y.: data curation; M.-D.W.: data curation and methodology; C.-W.Z.: data curation and supervision; W.Y.L.: conceptualization, supervision, and writing – review and editing; F.S.: conceptualization, methodology, supervision, and writing – review and editing; T.Y.: conceptualization, formal analysis, funding acquisition, methodology, supervision, and writing – original draft, review, and editing.

Conflicts of interest disclosure

There were no conflicts of interest.

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Guarantor

Tian Yang.

Data availability statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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